Effect of recovery period of mixture pasture on cattle behaviour, pasture biomass production, and pasture nutritional value

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Effect of pasture different recovery periods

Abstract

Pasture management that considers pasture growth dynamics remains an open question. Conceptually, such management must allow for grazing only after the recuperation of the pasture between two separate timely grazing periods when pasture reaches optimum recovery, as the first law of Voisin's Rational Grazing system **(VRG)**. The optimum recovery period **(ORP)** not only implies a pasture with better nutritional value and higher biomass yield, but one that also reduces the production of enteric methane **(CH_4)** to improve the grazing efficiency of cattle. Therefore, this study aimed to evaluate three different recovery periods **(RP)** of mixed grasses on the grazing behaviour of heifers, as well as herbage selectivity, herbage yield and nutritional value, *in vitro* degradability, and CH_4 production. Based on these criteria, three pasture RP of 24 **(RP24)**, 35 **(RP3)** and 46 **(RP46)** days were evaluated in six blocks using a randomized block design. At each predetermined RP, samples of the pasture were taken before the animals were allowed to graze. Right after collecting the pasture samples, heifers accessed the pasture during four consecutive hours for grazing simulation and behavioural observations. We also measured the bite rate of each animal. The pasture growing for 24 days had the highest biomass production, best nutritional value, best efficiency of *in vitro* CH_4 relative emission (ml) per DM degraded (g), and bite rate of the three RP. Heifers all selected their herbage, irrespective of RP, but with different nutritional value and higher *in vitro* degradability. However, this did not change the production of *in vitro* CH_4. Considering the growth conditions of the area where the study was performed, we recommend the shorter RP24 as the most suitable during the summer season. The study's findings support the idea of management intervention to increase the quality of grazing systems.

Keywords: Cattle behaviour, Environmental impact, Grazing, Management, Sustainability

Implications

The enteric methane (CH_4) is the most important greenhouse gas emitted from agriculture and ruminants raised in grazing systems are accepted as the bigger emitters, mainly in Tropical countries. However, the pasture management adopted in intensive grazing systems is shown to be an efficient strategy that influences the production of the gas. Controlling the period of pasture growth as well as the access of cattle to graze enables high nutritional value and productive pasture. This, therefore, will influence the grazing behaviour of cattle towards improving their performance and efficiency which promotes the reduction of environmental impacts like CH_4 emissions.

Introduction

Livestock is widely accepted as the biggest anthropogenic source of greenhouse gas **(GHG)** emissions. Enteric methane **(CH_4)** is the most important GHG and accounts for 40% of total GHG emissions from agriculture. Of this percentage 74% of CH_4 emissions comes from cattle (Faostat, 2016). Methane emissions are more prevalent in cattle fed pasture than in cattle fed grain, especially in tropical pastures (Archimède *et al*., 2018).

Most pasture-based countries have pastures composed of tropical species increasing even more CH_4 enteric emissions. Therefore, these countries have been criticized worldwide, jeopardizing the reputation of their livestock production. Nevertheless, most of the information that supports high emissions from grazing cattle comes from extensive systems in which the pasture is degraded and/or poorly managed and with low nutritional value (Berndt and Tomkins, 2013). Additionally, in extensive systems, animals are free to select their own grazing paths (Badgery *et al*., 2017). Consequently, they end up coming back to the same areas, which results in complicating soil restoration and impairing the radicular development systems of plants (Pulido *et al.,* 2018).

The improvement of pastures in tropical grass-based systems plays an important role in mitigating CH_4 gas emission due to its influence on herbage utilization and animal performance (Souza Filho *et al.,* 2019). Voisin's Rational Grazing **(VRG)** is an agroecological pasture management system widely used in countries of South America (see 1st, 2nd and 3rd Pan-American Meeting on Agroecological Pasture Management in: Cadernos de Agroecologia http://cadernos.aba-agroecologia.org.br/index.php/cadernos/issue/view/5). Following VRG, total pasture area is divided into paddock, in which the presence of the animal is based on the growth of monitored plant species which is controlled by the visual evaluation of herd management, thus differing from extensive systems. VRG is based on four laws of rational grazing (Voisin, 1974). One of these laws allows for the complete restoration of pasture reserves at the root level between two separate grazing periods. During such times, plants will grow and achieve high digestibility and accumulation of nutrients, as well as high DM yield per time and per area. This is called the optimum recovery period **(ORP)**, and it is also the designated time when animals should gain access to the paddock (Voisin, 1974).

In practice, VRG enables high nutritional value and productive pasture towards improving animal performance, including the reduction of environmental impacts like enteric CH_4 emissions (Stanley *et al.,* 2018). Moreover, VRG pastures are associated with high soil carbon **(C)** accumulation due to the increase biocenosis that increase and conserve the soil organic matter (Seó *et al.,* 2017) that helps reduce carbon dioxide **(CO_2)** concentration in the atmosphere and further mitigate GHG emissions

(Stanley *et al.,* 2018). Moreover, this system can reduce overgrazing, which potentially protects the soil against erosion and degradation.

When the recovery period **(RP)** is not managed correctly, plants might achieve maturity. Therefore, the vegetative phase ends, and the reproductive phase starts, and all the energy of the plant is directed toward flowering and seed formation. This maturation stage in pasture plants has, as a result, lower nutritional value than plants at earlier growth stages (Bhatta *et al.*, 2016). In later stages, the plant will have higher fibre and less protein concentration; and this typically promotes more production of enteric CH_4 (Jonker *et al.,* 2016). However, considering that the VRG pasture is composed of multi-species with different growth dynamics (Voisin, 1974; Machado Filho *et al*., 2014), animals should be able to select desired nutritional herbage (Wallis de Vries, 1995). However, in intensive systems, the animal is restricted from freely selecting their grazing areas (Badgery *et al.,* 2017) owing to the short time (i.e., 24 to 48-h) they spend in the paddock.

While improvement of pasture management is known to reduce CH_4 emissions, to the best of our knowledge, no study has yet reported the effects of different pasture RPs in the VRG system on animal selectivity and their CH_4 production. *In vitro* techniques in the laboratory reproduce the methanogenic potential of different diets, which allows the opportunity of a pre-evaluating before following up with *in vivo* techniques (Hill *et al.,* 2016). Therefore, we aimed to evaluate the effect of three different RP of mixed grasses on heifers' behaviour and herbage selectivity, herbage yield and nutritional value, as well as *in vitro* degradability and CH_4 production.

Material and methods

Site description

The study was undertaken during the summer between December of 2017 and February of 2018 at the VRG Unit of the Federal University of Santa Catarina Experimental Farm of Ressacada, Florianópolis, Brazil (27°40’25” S; 48°32’30” W). The land is flat, and the soil of the farm is classified as a Typical Hydromorphic Quartzic Neossolo (*Neossolo Quartzarênico Hidromórfico Típico*), consisting predominantly of dark sand, with low levels of phosphorus and potash, pH in water 5,5, a high content of organic matter and the groundwater level at less than 1m from the surface. The climate in this region is characterized as Cfa, i.e., subtropical humid, according to the Köppen climate classification (Álvares *et al.*, 2013). The annual average rainfall is 1462 mm, well distributed across the year, and the average temperature is 20 °C. During the experiment, no unexpected weather change was noticed.

The animals are routinely maintained on a 21 ha pasture platform divided into 84 paddocks averaging 2500 m^2 under a VRG management system. The pasture is mainly composed of native tropical species classified as C4. The main species identified were *Andropogon lateralis* Nees, *Axonopus affinis* Chase, *Axonopus obtusifolius* Raddi, and *Ischaemum minus* J. Presl. From the Poaceae family; *Eleocharis maculosa* (Vahl) Roem. & Schult, *Rhynchospora holoschoenoides* Heiter, *Rhynchospora tenuis* Link from Cyperaceae; *Juncos tenuis* Willd. from Juncaceae; and *Desmodium adscendens* (Sw.) DC. and *Desmodium incanum* DC. from Fabaceae. Although the pasture is fairly diverse, composition among paddocks is consistent. Animals were moved on a daily basis to a new paddock with mineral salt and water *ad libitum*.

Animals, treatments, and experimental design

Six paddocks from the VRG unit were used in a randomized block design to minimize possible differences on soil and vegetation among paddocks. Every paddock (block) was then divided into three plots (834 m²) in which three different RPs were established: **RP24**: 24 days, **RP35**: 35 days, and **RP46**: 46 days. In each block (paddock), each RP was randomly assigned to a given plot. These periods were chosen to represent the average RP of 35 days ± 11 days used at the experimental farm.

Eighteen heifers (15 Jersey, 1 Holstein, and 2 Jersey × Holstein) were selected and separated into three groups according to weight and to social hierarchical ranking of the herd to which they belonged, each having an average weight of 300 ± 36.07 (SD) kg. Groups of heifers were scheduled to be used in accordance with a systematic distribution and the pre-determined RP. The groups went through all the blocks according to the RP in a 3x3 double Latin Square design.

Measurements

Pasture collection and samples. At the beginning of the experiment, the blocks were trimmed at about 2 cm from soil level on specific days in order to allow the pasture to grow to a predetermined RP (24, 35, and 46 days) and the scheduled organization of the sample collections. At each predetermined RP of the pasture, samples of the available biomass were taken of the pasture before that the animals were allowed to graze. At soil level, five random samples were cut using a 0.5 x 0.5 m² square. Each sample was weighed to measure biomass production and then composed as a final sample for bromatological analysis. By dividing the total biomass production over number of days of each RP, we determined biomass daily growth of each RP.

Samples were weighed, taken to a laboratory, dried at 55ºC for 72-h, and then weighed to determine DM (AOAC, 1995). After drying, samples were ground to one mm in a hammer mill for further analysis.

Ash concentration was estimated according to the AOAC (1995) methods. For Crude Protein (CP), Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF), Near Infrared Reflectance Spectroscopy (NIRS) was used. The reflectance spectra of samples were collected with an MPA FT-NIR spectrometer (BRUKER OPTIK GmbH, Rudolf Plank Str. 27, D-76275 Ettlingen) in triplicate. The spectral area of 3600 to 12500 cm^{-1}, accumulating after 64 scans, accounts for the bromatological values estimated by least square means regression calibration curve obtained by an OPUS 7.5 (PLS) (Bjorsvik and Martens, 2001).

Behavioural observations and hand-plucking. Right after collecting the pasture samples at each scheduled RP, the groups of heifers accessed the plots during four consecutive hours for grazing simulation and behavioural observations. Grazing simulation was performed according to the hand-plucking technique (Wallis de Vries, 1995), and three subsamples were collected from each animal per grazed plot in order to compose a representative sample from each group per block per RP. Samples collected by hand-plucking were used for the same bromatological analysis as previously described.

The observations were carried out during 4-h consecutively. Observers were previously trained and were located 15 m from the animals to reduce disturbance (Machado Filho *et al.,* 2014). A sample scan from each animal was taken every 10 min (Altmann, 1974). The following behaviours were recorded: grazing, ruminating, idling, and any other behaviour of interest. Additionally, we measured the bite rate of each animal five times each hour for 30 seconds each, and then one value per group was calculated.

In vitro gas production technique. Gas production and the *in vitro* degradability of pasture samples, as well as the hand-plucking samples, were analyzed at the Analytical Chemistry Plant Laboratory (LQAP) from Embrapa Cerrados, located in Planaltina-DF, using the methodology previously described by Mauricio *et al*. (1999). Briefly, a 0.5 g sample of each treatment was duplicated and weighed in F57 Ankom bags. These bags were introduced in 100 ml amber fermentation bottles. The bottles were kept sealed with a silicone cap and an aluminium seal at 39ºC until inoculation. The culture media were prepared following the recommendation of Menke *et al.* (1979). After the mixture of reagents, the solution was infused with CO_2 for 2-h, followed by calibration of pH to 6.9. Rumen fluid was collected from two four-year-old male steers with an average weight of 400 kg. One was a Nelore and the other was a three-quarter Gir-Holstein. Both were kept under *Urochloa brizantha (*Hochst *ex A. Rich.* cultivated by Marandu) grazing systems with at least 10 kg of DM/kg liveweight per day allowance, including *ad libitum* water and mineral supplemented with at least 65g/kg of phosphorus and 2 kg/d of concentrate (80% of total digestible nutrients **(TDN)**and 12% of CP). The use of two different male guarantee better representativeness of the inoculum, and there is no problem in using males instead of heifers, as the focus was evaluating the degradability of feedstuffs rather than animals' performance. In this case, whenever is possible, the inoculum should be obtained from donors fed with similar diets than the feedstuff is being tested (forage) (Mauricio *et al*., 1999). The rumen fluid sample was taken through a vacuum system in which a hose with small holes for filtration was introduced into the bull's cannula.

The material was stored in a thermos bottle. At the LQPA, both samples were mixed together, filtered again, using cheesecloth, and then added to the culture media. This procedure was done using a water bath control of 39ºC and a constant flow of CO_2. After mixing, the ruminal fluid of the culture media filled 50 ml of solution in each bottle which consisted of 36.4 ml of culture media and 13.6 ml of ruminal fluid. We then put the bottles in an oven at 39ºC for 48-h.

The gases produced were measured at 6, 24, and 48-h by the displacement of water measured by the apparatus of communicating vessels described by Fedorah and Hrudey (1983). During these measurements, bottles remained immersed in a 39°C water bath. The volume of gas produced was recorded, and a sample of gas was collected through a syringe and injected into vacuum Exetainer® vials (Labco Limited). To quantify *in vitro* degradability, F57 bags were drained from the bottles after 6, 24, and 48-h. They were washed with cold tap water, dried at room temperature for two days, baked at 105ºC for 4-h in the oven, and then weighed.

CH_4 measurements. Vials with gas samples were sent to Embrapa Dairy Cattle – Juiz de Fora/MG for the reading of fermentable gases. Samples were read in gas chromatography equipment (03 CG-FID, Agilent Technologies 7820ª) with EzChrom Elite interface software, equipped with 2 6-way valves. One was used for the sampler and the other one as selector, allowing the constituents to pass, or not, through the second column. Hydrogen **(H_2)** was used as a carrier gas on a flux of 8.3ml/min. The chromatography calibration was realized with reference standard certificated by Linde Industry on concentrations of CH_4: 5.05; 10.2; 14.7; 20.1 and CO_2: 20.2; 39.7; 58.3; 79.9.

Statistical analysis

Statistical analyses were performed in R (R Core Team, 2018) using R packages lme4 (Bates *et al.,* 2015). Mixed-effects linear models were fitted to assess the effect of the three RP (24, 35 or 46 days) and method of grass sampling (hand-plucking and square) on each measured variable (CP, NDF, ADF, Ash, Biomass daily growth, DM, CH_4 production, and pasture degradability). The plots were the experimental unit and the blocks were considered as a random effect. We investigated the associations between the heifer's grazing frequency and the pasture RPs (24, 35 or 46 days) through mixed-effects binary logistic regression (Bernoulli) (Korner-Nievergelt et al., 2015); the effect of the pasture RPs on bite rate was evaluated by mixed-effects linear regression. Date and heifers nested within each date were used as random effects to account for pseudo replication. The group was considered the experimental unit. The results are presented as estimated marginal means ± SEM. Model assumptions were adjusted graphically for normal distribution and homoscedasticity of the residuals. P values were obtained by Wald X^2 test type II (P < 0.05 or P < 0.01).

Results

Table 1 shows the estimated means for bromatological composition evaluated for the different pasture RPs and the ability of heifers to select their herbage. Ash and CP decreased from RP24 to RP46 (P < 0.05), while NDF was lower for RP24 compared to the other RPs (P < 0.01). We noticed no difference for ADF among the RPs (P = 0.86). Biomass available at the moment heifers accessed the paddocks was higher (P = 0.003) for RP3 (1582.4 kg DM/ha) comparing to RP1 (1029.6 kg DM/ha) and RP2 (1102.5 kg DM/ha), but daily growth was higher when cutting interval was 24 days (*P* = 0.01). We found no interaction for the different RPs and the method of collecting pasture samples (*P* > 0.05). However, the pasture selected by heifers had higher CP and less ADF and NDF proportion than the pasture offered on paddocks (*P* < 0.05).

The *in vitro* degradability of the pasture was similar among the RPs evaluated (*P* = 0.07); nonetheless, the herbage selected by heifers was more degradable than the pasture offered in the paddocks in all three RPs (*P* < 0.001, Figure 1). The CH_4 production per DM degraded each hour of incubation is shown in Table 2. During the shorter RP (24 days), the pasture had better efficiency of *in vitro* CH_4 relative emission (ml) per DM degraded (g) (*P* < 0.01). However, we did not observe a difference between the pasture offered and the pasture selected for CH_4 production in any RP tested (*P* = 0.52).

Behavioural observations are shown in Table 3. Heifers grazed most of the time during the four hours of observation in the paddocks. The higher frequency of grazing occurred on pasture with 46 days of RP (*P* < 0.001), and the bite rate was higher when heifers grazed the shorter RP24 (*P* < 0.001).

Discussion

Generally, when compared to the other RPs, RP24 had higher biomass production, better nutritional value, better efficiency of *in vitro* CH_4 relative emission (ml) per Kg of degraded DM, and heifers grazing on RP24 had higher bite rate, suggesting higher ingestion. RP35 was intermediate between RP24 and RP46 only for CP and ashes, without differences between them. Nevertheless, the higher NDF in RP35 and RP46 compared to RP24 stimulated the increase of *in vitro* CH_4 production per gram of degraded DM for both, RP35 and RP46. Thus, it is expected that the high nutritional value of feed leads to a better fermentative parameter in the rumen and generates more end products, as volatile fatty acids **(VFA)**, which are the main energy source for ruminants (Johnson and Johnson, 1995). However, the proportion of VFA produced depends on the chemical profile of the digested feed, which, in turn, affects the amount of CH_4 that will be produced, although we could not evaluate VFA proportions in this study.

The fibre benefits cellulolytic bacteria in the rumen; such bacteria specialize in degrading components of the cell wall. As a result, a higher acetate is produced, rather than propionate, which leads to a higher amount of H_2 free in the rumen, becoming, in turn, the substrate for methanogenic bacteria to produce CH_4 (McGeough *et al.,* 2019). In this study, the pasture was mainly composed of C4 plant species. Although we didn't classify the pasture consumed by the animals, it was possible to observe that most of the animals' consumption were C4 plants, which naturally have thick cell walls with high fibre, high degree of lignification, and minor concentrated nutrients that will be digested, which consequently promote large amounts of CH_4 gas (Archimède *et al.,* 2018).

C4 species have a high rate of photosynthetic ability that yields high DM potential (Silva *et al*., 2015). When these plants grow in adequate conditions, their growth is favoured, and these characteristics are aggravated. Therefore, as plant growth advances, their chemical profile changes (Bhatta *et al.,* 2016). The length of the recovery period is negatively correlated with the digestibility of fibre, and more acetate is produced (Jonker *et al.,* 2016), creating more CH_4. On the other hand, the low NDF proportion shifts fermentation towards propionate production (McGeough *et al.*, 2019; Johnson and Johnson, 1995) reducing substrate availability for methanogenic bacterial activity.

Similarly, it was expected that RP46 would have higher in vitro CH_4 production than RP35, considering its advanced pasture maturity and corresponding reduction in protein and ash proportion. However, their fibre concentration was similar, as well as their CH_4 production. The *in vitro* gas production technique indicates the readily available fermentable substrate that rumen microorganisms will use as energy source to produce VFA, and the rate that the feedstuff will be degraded (Mauricio *et al.,* 1999). Pasture with low nutritional value are slowly fermented and yield more acetate than propionate. We did not evaluate more than 48-h of incubation, so we cannot make inferences about the kinetics of fermentation. High nutritional value feed might also result in greater CH_4 production, depending of its digestibility, carbohydrate profile and the proportion of VFA produced (McGeough *et al.,* 2019). However, feed intake would most likely increase, which would reduce CH_4 per kg of DM intake more than feed of low nutritional value (Johnson and Johnson, 1995). Warner *et al*. (2016), evaluating three stages of maturity of grassland, observed a decrease in daily gross production of CH_4 from cows on longer cutting intervals. Nevertheless, when considering the emissions per kg of organic matter intake, the authors observed that increased CH_4 production correlated with plant maturation. To explain, for shorter pasture cut times, animal intake is increased, and CH_4 emissions are diluted, while on longer pasture cut times, the low daily gross production of CH_4 is not compensated by the reduction in animal intake. The main species from the grassland studied in Warner *et al.* (2016) was ryegrass, a C3 species, the maturation rate of which is slower than C4 species. Nonetheless, by the reduction in nutritional value from RP24 to RP46, we can speculate that the animals would consequently also reduce their intake, as the stage of maturity advances, and we would notice an increase in the intensity of CH_4 emissions (kg of CH_4/kg of DM intake), according to this advance. While RP46 and RP35 had similar CH_4 emission, RP46 had poorer nutritional value; therefore, RP46 is expected to have a higher intensity of CH_4 emissions. Meanwhile, the higher feed intake would compensate for the intensity of RP35 CH_4 (McGeough *et al*., 2019 (Johnson and Johnson, 1995) and the low CH_4 production in RP24 would be diluted even more.

RP24 was more productive than RP35 and RP46 with higher biomass production per day. Shorter RP has been associated with more resilient pasture, thereby increasing efficiency and the number of grazing cycles (Silveira *et al.,* 2013). Shorter RP is found to be sufficient for the accumulation of carbohydrates and plant restoration, resulting in minor dead material proportionally and better leaf-stem relationship for grazing (Chapman *et al*., 2014). This relationship positively affects plant growth, promoting better cell content and better nutritional value (Moura *et al*., 2017). While fibre is expected to increase and protein is expected to decrease in long periods of pasture growth, shorter regrowth intervals (under 30 days) are associated with a greater leaf-stem ratio with minor cell wall fraction and major DM soluble fraction (Moura *et al*., 2017). Furthermore, higher biomass production leads to higher C stock in soil. RP24 resulted in less efficiency of CH_4 relative emission, but it is also assumed to have had higher ability in sequestering C (Silva *et al*., 2016; Stanley *et al.,* 2018).

It is also important to consider the composition of the sward. C4 species present a high rate of senescence, mobilizing nutrients faster for their growth (Silva *et al.,* 2015). Thus, since they were in an adequate environment and climate, their growth and maturation were favoured and accelerated. On the other hand, when areas have low productivity, short RP might not be enough for plant recovery (Badgery *et al*., 2017). Since many factors influence pasture dynamics, the scenario in which the pasture is established is very important to its composition and growth, as are management and species characteristics per se (Badgery *et al.,* 2017). For that reason, it is difficult to define a fixed RP, especially when the pasture is a mixture. This accounts for the variability of RP in the VRG system and explains why RP must be defined from time to time based on species with the greatest interest (Voisin, 1974). Following this VRG law, determining the pasture's ideal RP in the context of the control of pasture growth and plant species growth dynamics can result in improving productivity and nutritional value. Taking the present study as an example, 35 days is the average RP used at the Experimental Farm of Ressacada, the study site; nevertheless, considering the predominance of C4 species during the summer season and the results obtained in this study, 24 days of RP seem to be the best option for the season. For other seasons, like fall and winter, we may have an "optimum" RP interval even longer than the average 35 days. The region has a well-defined warm and cold season, with well distributed rainfall across the year (Álvares *et al*., 2013). During the experiment, no unexpected weather change was noticed. The seasonal climate changes influences plants growth. This is an ultimately reason why RP should vary over the seasons. Although this study hasn't been conducted over multiple growing seasons, as would be ideal, we know from a number of other studies that RP is highly variable according to season, soil fertility, plant species composition, among other factors, as well documented (see 1st, 2nd and 3rd Pan-American Meeting on Agroecological Pasture Management in: Cadernos de Agroecologia http://cadernos.aba-agroecologia.org.br/index.php/cadernos/issue/view/5). Moreover, it is not the aim of this study to determine an ideal RP, but to better understand how different RPs can affect herbage quality, production and enteric emissions. Variable RP among paddocks and seasons is a characteristic of the VRG (Voisin, 1974). Further research is required to be conducted over multiple growing season to better understand how RP for each season varies.

Pasture management is the gold standard for increasing the sustainability of grazing systems. While we showed the best RP to reduce the CH_4 emission from cattle, other studies have shown that plants from well-managed pastures also have the ability to sequester C from the atmosphere during photosynthesis (Seó *et al.,* 2017; Stanley *et al.*, 2018). When the amount of C emitted is lower than the amount that is being accumulated in soil by plant roots, we have a positive balance of C, and the livestock emissions are indeed offset (Silva *et al*., 2016). The different rates of C balance between the different livestock systems illustrate that intensive grazing systems, like VRG, can mitigate GHG emissions from livestock by adopting the best management practices (Stanley *et al.,* 2018; Souza Filho *et al.,* 2019).

Intensive grass-based systems, like VRG, are found to reduce animal selectivity (Badgery *et al.,* 2017). Despite that, herbage selected by heifers was higher in protein, degradability rate and lower in fibre in all the three RPs. The same was observed by Machado Filho *et al.* (2014) who reported that grazing cows in a VRG system compensated for the low levels of protein from supplement that they received through the selection of plants with better protein value. Taking into account that our paddocks were composed of ample species diversity, heifers could select species to meet their nutritional requirements. Animal selectivity is also connected to the frequency of grazing behaviour and herbage mass offered. Cows are tempted to spend more time in grazing when more plant biomass is available (Motupalli *et al.,* 2014). RP24 was more productive than RP35 and RP46 with higher biomass production, considering the period that the pasture was allowed to grow. However,

the biomass available at the moment that heifers accessed the paddocks was higher in RP46 (1582.4 kg DM/ha) compared to RP35 (1102.5 kg DM/ha) and RP24 (1029.6 kg DM/ha), since RP46 had more days of pasture growth. Nevertheless, grazing involves the search for herbage, not just DM intake per se. In RP46, heifers grazed for longer time, but had a lower bite rate than they did in RP24 and RP35. Considering the poorer nutritional value, we can suppose that heifers were more selective when in RP46, compared to RP24 and RP35, in order to achieve a more satisfactory pasture with better nutritional value amenable to their preference. Moreover, the higher NDF concentration in RP46 required the animals to devote more effort in harvesting and chewing this pasture, which agrees with the lower bite rate in this RP. This might indicate that the intake most likely was reduced in RP46, thus reinforcing the idea that heifers have higher intensity of CH_4 emission when grazing on longer RP, as previously stated. In contrast, in RP24, heifers increased bite rate as a strategy to compensate for pasture structure variations and probably to increase the volume of forage intake (Mezzalira *et al.,* 2014), demonstrating an inverse relationship between bite rate and mass volume. Although we did not measure plant height, it most likely varied among the three RPs tested, considering the different time plants were given to grow. Since heifers grazed an equal amount of time when in RP24 and RP35, the higher bite rate in RP24 suggests a higher intake when heifers were in this treatment. Moreover, methane production was higher in RP35 than RP24 (Table 2). Thus, heifers grazing in the RP35 pasture probably emitted more CH_4/kg of DM intake than when grazing in the RP24 pasture.
In summary, the shorter RP24 seems to be the most suitable RP for the C4 species in the area where the study was done during the summer. Further research is needed to understand the best RP in other seasons. Heifers apparently adjusted

their grazing behaviour according to the different RPs. This study supports the idea of adequate pasture management and variable RP in order to increase herd grazing efficiency and provide a better quality grazing system. Furthermore, the study indicates the cows' ability to select their own herbage when needed and their compensatory strategies in the context of variable pasture structure.

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Declaration of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statement

The study was performed in accordance with the requirements of the Ethics Committee on Animal Use of the Federal University of Santa Catarina (CEUA/UFSC) under the approved protocol number 1004100516.

Software and data repository resources

All the data in the R script for the statistical analyses for the current manuscript is public available at: http://doi.org/10.5281/zenodo.3520917

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Table 1 *Biomass daily growth and composition of pasture growing during different recovery periods: 24 days, 35 days, and 46 days, considering the pasture offered on the paddock and the pasture selected by the heifers.*

	Recovery period (days)	Mean	SEM	P-value	Random Effect (SD)[1] Block
Biomass daily growth	24	42.9^{a}	3.62	*	5.33
Kg of DM per ha per day	35	31.5^{b}	4.08		
	46	34.4^{b}	4.08		
CP (% of DM)	24	9.7^{a}	0.76	*	1.66
	35	9.2^{ab}	0.51		
	46	8.2^{b}	0.51		
	Offered	9.0^{A}	0.76	***	1.66
	Selected	10.7^{B}	0.51		
NDF (% of DM)	24	57.1^{B}	1.11	***	0.66
	35	63.7^{A}	1.53		
	46	62.4^{A}	1.53		
	Offered	60.9^{A}	1.11	**	0.66
	Selected	58.1^{B}	1.53		
ADF (% of DM)	24	35.9	0.94	0.86	1.91
	35	36.4	0.76		
	46	37.2	0.76		
	Offered	36.5^{A}	0.94	***	1.91
	Selected	34.3^{B}	0.76		
Ash (% of DM)	24	7.6^{A}	0.31	***	0.68
	35	7.6^{AB}	0.21		
	46	6.8^{B}	0.21		
	Offered	7.4	0.31	0.17	0.68
	Selected	7.2	0.21		
DM (%)	24	19.4	0.84	0.45	1.34
	35	19.2	0.90		
	46	20.3	0.90		
	Offered	19.6	0.84	0.33	1.34
	Selected	20.1	0.90		

[1] Block (n=6) was included as random effect, SD of average variation of each variable (CP, ADF, NDF, Ash and Yield) previewed from model is presented for each level of random effect.

[a,b] Values within a row with different superscripts differ significantly at *P*<0.05.

[A,B] Values within a row with different superscripts differ significantly at *P*<0.01.

*, ** and *** indicate probabilities levels at *P*<0.05, *P*<0.01 and *P*<0.001, respectively.

Table 2 *Cumulative in vitro production of methane (CH_4) gas (ml) per DM degraded (g) from pasture that was offered to the heifers, growing during different recovery periods: 24 days, 35 days, and 46 days, according to the incubation time (6, 24 and 48-h).*

	24 days	35 days	46 days	SEM	P-value
6-h	0.1^{A}	0.7^{B}	0.6^{B}	0.18	**
24-h	4.0^{A}	6.4^{B}	5.1^{B}	0.56	***
48-h	9.6^{A}	12.3^{B}	10.5^{B}	0.68	***

^{A,B} Values within a row with different superscripts differ significantly at P<0.01.

** and *** indicate probabilities levels at P<0.01 and P<0.001, respectively.

Table 3 *Frequency of grazing behaviour (%) and bite rate per minute of heifers in relation to different recovery periods of pasture: 24 days, 35 days, and 46 days.*

	24 days	35 days	46 days	SEM	P-value
Grazing (%)	63.5^{B}	64.3^{B}	66.5^{A}	0.09	***
Bite rate per minute	44.4^{A}	36.8^{B}	37.2^{B}	1.34	***

^{A,B} Values within a row with different superscripts differ significantly at P<0.01.

*** indicate probabilities levels at P<0.001

Figure captions

Figure 1 Herbage selected by heifers (solid line) had higher pasture DM degradability (%) than herbage offered on paddocks (dotted line) at all three points of fermentation evaluated (6, 24, and 48-h). *P*<0.05.